

WHAT IS CLAIMED IS:

1. A DNA micro-array for detecting nucleic acid molecules having target base sequences in a sample comprising a substrate and nucleic acid probes having
5 base sequences substantially complementary to the target base sequences immobilized on the substrate, wherein the array contains additional probes of one or several kinds selected from the following probes:
probes for internal standard nucleic acids of one or
10 several kinds which hybridize with said internal standards and are added for quantitative evaluation of PCR of said nucleic acid molecules having the target base sequences;
probes for external standard nucleic acids of one or
15 several kinds which hybridize with said external standards and are added for evaluation of accuracy of detection operation and for quantitative analysis of the amount of the probes having base sequences substantially complementary to the target base
20 sequences;
and probes added for quantitative evaluation of the amount or density of said nucleic acid probes having base sequences substantially complementary to the target base sequences, which are immobilized by the
25 same method as said nucleic acid probes.

2. A DNA micro-array for detecting nucleic acid

molecules having target base sequences in a sample comprising a substrate and nucleic acid probes having base sequences substantially complementary to the target base sequences immobilized on the substrate,
5 wherein the array contains additional probes of one or several kinds selected from the following probes: probes for internal standard nucleic acids of one or several kinds which hybridize with said internal standards and are added for quantitative evaluation
10 of PCR of said nucleic acid molecules having the target base sequences;

probes for external standard nucleic acids of one or several kinds which hybridize with said external standards and are added for evaluation of accuracy
15 of detection operation and for quantitative analysis of the amount of the probes having base sequences substantially complementary to the target base sequences;

in which said internal and/or external standard
20 nucleic acids are added in order to quantitatively determine a concentration of the target nucleic acid molecules in the sample.

3. The DNA micro-array according to claim 2,
25 wherein at least the internal standard probes or the external standard probes or both are immobilized on the substrate as a group of at least four levels of

amount or density.

4. The DNA micro-array according to claim 2,
wherein the internal standard probes include at least
5 two probes corresponding to PCR products with
different chain lengths derived from the internal
standard nucleic acids.

5. The DNA micro-array according to claim 2,
10 wherein the external standard probes include at least
two probes each having a mutually different base
sequence of any length each complementary to the
external standard nucleic acids added.

15 6. The DNA micro-array according to claim 2,
wherein the internal standard probes and the external
standard probes are synthetic nucleic acids
immobilized on the substrate.

20 7. The DNA micro-array according to claim 6,
wherein the synthesized nucleic acid has a chain
length of 15 to 75 bases.

8. A primer set for PCR of internal standard
25 nucleic acids to be amplified together with target
nucleic acids during an PCR of the nucleic acids
having the target base sequences upon quantitatively

detecting the nucleic acids having the target base sequences using a DNA micro-array, wherein

chain lengths of PCR products derived from the nucleic acids having the target base sequences are
5 designed to be substantially equal to chain lengths of PCR products derived from the internal standard nucleic acids given by the primer set.

9. A kit for detecting a target base sequence,
10 which contains a primer set for PCR of an internal standard nucleic acid to be amplified together with a target nucleic acid during PCR of the nucleic acid having the target base sequence upon quantitatively detecting the nucleic acid having the target base
15 sequence using a DNA micro-array, comprising at least two of the primer sets according to claim 8 corresponding to different chain lengths when an amplified product derived from the nucleic acid having the target base sequence has at least two
20 chain lengths.

10. The kit for detecting a target base sequence according to claim 9, wherein the primer sets include at least one primer set for an amplified
25 product chain length of 200 bp or less, at least one primer set for an amplified product chain length of 200 to 500 bp, at least one primer set for an

amplified product chain length of 500 to 2,000 bp and at least one primer set for an amplified product chain length of 2,000 bp or more.

5 11. A kit for detecting a target base sequence, which contains external standard nucleic acids to be added to a sample upon quantitatively detecting a target nucleic acid using a DNA micro-array, comprising at least two external standard nucleic
10 acids which are synthesized nucleic acids labeled with a detectable marker.

 12. The kit for detecting a target base sequence according to claim 11, wherein the marker
15 comprises a fluorescent material, a radioactive material or a light emitting material.

 13. A kit for detecting a target base sequence, which contains internal standard nucleic acids to be
20 amplified together with a target nucleic acid during PCR of the nucleic acid having the target base sequence upon quantitatively detecting the nucleic acid having the target base sequence using a DNA micro-array, comprising at least two nucleic acids
25 derived from microorganism or virus as internal standard nucleic acids having no homology with the target base sequence to be detected.

14. A DNA micro-array having the first set of nucleic acid probe dots including a plurality of target nucleic acids arranged in a matrix pattern on a substrate, further comprising the second set of
5 nucleic acid probe dots for assay of amounts or a densities of the nucleic acids in said dots, which are formed by the same method as the formation of said first set of nucleic acid probe dots and arranged on part of a surface of the substrate having
10 said second set of nucleic acid probe dots formed thereon and whose average nucleic acid density per dot is determined.

15. The DNA micro-array according to claim 14,
15 further comprising a plurality of said second set of nucleic acid probe dots having different levels of average nucleic acid density with average nucleic acid density per dot determined as the nucleic acid probe dots for use as density standards.

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16. The DNA micro-array according to claim 14, wherein the average nucleic acid density per dot of the nucleic acid probe dots having the determined average nucleic acid density per dot are determined
25 by chemical analysis separately.

17. The DNA micro-array according to claim 16,

wherein inductively coupled plasma mass spectrometry (to be abbreviated as ICP-MS hereinafter) is used for the chemical analysis for determining the average nucleic acid density per dot.

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18. The DNA micro-array according to claim 14, wherein the nucleic acid probe comprises a single-stranded nucleic acid.

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19. The DNA micro-array according to claim 14, wherein the nucleic acid probe including a single-stranded nucleic acid and a target nucleic acid introduced by hybridization of the nucleic acid probe are both existent on the substrate.

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20. An analyzing method for a DNA micro-array having nucleic acid probe dots including a plurality of nucleic acids arranged in a matrix pattern on a substrate, characterized in that:

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on part of a surface of the substrate having said second set of nucleic acid probe dots formed thereon, nucleic acid probe dots whose average nucleic acid density per each dot has been determined are formed as density standards by the same method as the formation of said first set of nucleic acid probe dots, where the density standard nucleic acid probe dots are a plurality of nucleic

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acid probe dots having different levels of average nucleic acid densities, whose average nucleic acid density per each dot has been determined; and

a nucleic acid concentration of the first set

5 of nucleic acid probe in each dot having an undetermined concentration arranged on the substrate is determined by the secondary ion mass spectrometry by using a calibration curve drawn based on signal intensities of secondary ions detected when secondary

10 ion mass spectrometry is carried out on the plurality of said second set of nucleic acid probe dots having the different levels of average nucleic acid densities, .

15 21. The analyzing method for a DNA micro-array according to claim 20, wherein time-of-flight type secondary ion mass spectrometry is used as the secondary ion mass spectrometry.

20 22. The analyzing method for a DNA micro-array according to claim 21, wherein a secondary ion intensity detected by the secondary ion mass spectrometry is an integral intensity (count value) of specific secondary ions derived from the nucleic

25 acid probes and released from a fixed area applied with primary ions when a dose of the primary ions is set to a fixed value of $1 \times 10^{14}/\text{cm}^2$ or less.

23. The analyzing method for a DNA micro-array according to claim 21, wherein a secondary ion intensity detected by the secondary ion mass spectrometry is an integral intensity (count value)
5 of specific secondary ions derived from the nucleic acid probes and released from a fixed area applied with primary ions when the dose of the primary ions is set to a fixed value of $1 \times 10^{12}/\text{cm}^2$ or less.

10 24. The analyzing method for a DNA micro-array according to any one of claims 21 to 23, wherein the secondary ions detected by the secondary ion mass spectrometry include an anion obtained by eliminating one hydrogen atom from a base of one of adenine,
15 thymine, guanine, cytosine, and uracil, or an anion selected from the group consisting of P^- , PO^- , PO_2^- and PO_3^- as the secondary ion derived from the nucleic acid probe.

20 25. The analyzing method for a DNA micro-array according to claim 21, further comprising displaying a detection result as an image which shows secondary ion intensity two-dimensionally according to an application position of the primary ions, based on
25 the secondary ion intensity detected by the secondary ion mass spectrometry.

26. A method of producing a DNA micro-array having nucleic acid probes arranged in a matrix pattern on a substrate, comprising, upon forming said second set of nucleic acid probe dots for use as
5 density standards whose average nucleic acid density per each dot is determined on part of a surface of the substrate having said first nucleic acid probe dots formed thereon:

forming said first set of nucleic acid probe
10 dots in the matrix pattern on the substrate; and

forming the second set of nucleic acid dots on the part of the surface of the substrate by the same method as the formation of said first set of nucleic acid probe dots , wherein the nucleic acid probe dots
15 for use as the density standards whose average nucleic acid density per each dot is pre-determined.